Persoonia 22, 2009: 1–13
www.persoonia.org

RESEARCH ARTICLE

doi:10.3767/003158509X415450

Phytophthora multivora sp. nov., a new species recovered from declining *Eucalyptus*, *Banksia*, *Agonis* and other plant species in Western Australia

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Key words

decline
dieback
forest
jarrah
phylogeny
Phytophthora citricola
tuart

Abstract A new *Phytophthora* species, isolated from rhizosphere soil of declining or dead trees of *Eucalyptus gomphocephala*, *E. marginata*, *Agonis flexuosa*, and another 13 plant species, and from fine roots of *E. marginata* and collar lesions of *Banksia attenuata* in Western Australia, is described as *Phytophthora multivora* sp. nov. It is homothallic and produces semipapillate sporangia, smooth-walled oogonia containing thick-walled oospores, and paragynous antheridia. Although morphologically similar to *P. citricola*, phylogenetic analyses of the ITS and *cox*1 gene regions demonstrate that *P. multivora* is unique. *Phytophthora multivora* is pathogenic to bark and cambium of *E. gomphocephala* and *E. marginata* and is believed to be involved in the decline syndrome of both eucalypt species within the tuart woodland in south-west Western Australia.

Article info Received: 21 October 2008; Accepted: 3 December 2008; Published: 2 February 2009.

INTRODUCTION

The oomycete genus *Phytophthora* includes many well-known species that contribute to and often drive tree declines worldwide. Knowledge about the diversity and significance of *Phytophthora* species in forest ecosystems has significantly increased in recent years as research has focussed on new and devastating tree declines in natural ecosystems in Europe and the Americas (Jung et al. 1999, 2000, 2002, Vettraino et al. 2001, 2002, Rizzo et al. 2002, Jung & Blaschke 2004, Brasier et al. 2005, Balci et al. 2007, Greslebin et al. 2007, Jung 2008) and advances in molecular techniques have improved our phylogenetic understanding of the genus (Cooke et al. 2000, Kroon et al. 2004). Since the discovery of *P. cinnamomi* in the south-west of Western Australia (WA) (Podger et al. 1965), this introduced pathogen has become renowned for its unparalleled impact on flora biodiversity with 40 % of the 5 710 species in the south-west Botanical Province found to be susceptible and 14 % highly susceptible (Shearer et al. 2004).

As a result of the wide scale forest quarantine and management of *P. cinnamomi* in WA, extensive and regular testing of soil and plant tissue samples for *P. cinnamomi* at the Vegetation Health Service (VHS) laboratory of the Department of Environment and Conservation has led to the isolation of a large range of *Phytophthora* spp. and undescribed *Phytophthora* taxa (Stukely et al. 1997, 2007a, b, Burgess et al. In press). The recovery of *Phytophthora* taxa other than *P. cinnamomi* from some sites with declining vegetation in WA has recently focussed attention onto their role in the decline of these woodland and forest ecosystems (Shearer & Smith 2000).

Across the south-west of WA there are a number of recently observed and significant forest declines occurring. In particular, the declines of *Corymbia calophylla* (Paap et al. 2008), *Eucalyptus wandoo* (Hooper & Sivasithamparam 2005), *E. gomphocephala* (tuart, Fig. 1a, b), *E. marginata* (jarrah, Fig. 1c, d), *E. rudis*, *Agonis flexuosa* and *Banksia* spp. (Fig. 1e, f) are causing concern to land managers and the general community. Within the tuart woodland of Yalgorup National Park on the Swan Coastal Plain south of Perth, a significant decline and substantial numbers of deaths of *E. gomphocephala* have been observed together with localised declines and mortality of *E. marginata* since the 1990s and *A. flexuosa* since 2006. A range of biotic and abiotic factors has been shown to contribute to tuart decline (Edwards 2004, Archibald 2006), although as yet, no satisfactory aetiology has been established. The progressive canopy thinning and dieback, and the heterogeneous distribution of the decline, are similar to Jarrah dieback (Shearer & Tippett 1989) and suggest the potential involvement of a *Phytophthora* species.

In May and June 2007 *Phytophthora* isolates were recovered from the rhizosphere of declining *E. gomphocephala*, *E. marginata* and *A. flexuosa* in Yalgorup National Park. These isolates morphologically resembled *P. citricola*, which has been recovered over the past three decades throughout the south-west of WA by the VHS (Stukely et al. 1997). However, recent re-evaluation of the VHS culture collection using molecular techniques has identified most of these isolates as a new taxon (*Phytophthora* sp. 4) in the *P. citricola* complex (Burgess et al. In press). DNA sequence data from the internal transcribed spacer regions (ITS1 and ITS2) and 5.8S gene of the rRNA operon and the mitochondrial *cox*1 gene were used in combination with morphological and physiological characteristics to characterise these isolates and compare them to the ex-type isolate of *P. citricola* as described by Sawada (1927). Due to their unique combination of morphological and physiological characters, and ITS and *cox*1 sequences, these semipapillate homothallic isolates from the south-west of WA are described here as a new species, *P. multivora* sp. nov.

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Fig. 1 a. Severe dieback and mortality of a forest stand of *Eucalyptus gomphocephala*; b. crown symptoms of a declining *E. gomphocephala* including thinning, clustering of leaves, and dieback of branches and parts of the crown; c. dieback and mortality of a forest stand of *E. marginata*; d. crown symptoms of a declining *E. marginata* including thinning, clustering of leaves and dieback of branches and parts of the crown; e, f. collar rot of *Banksia attenuata* caused by *Phytophthora multivora*; e. sudden wilting and death due to the girdling of the collar; f. tongue-shaped, orange-brown necrosis of the inner bark.

MATERIAL AND METHODS

Sampling and Phytophthora isolation

Phytophthora isolates sampled from the tuart forest were obtained using soil sampling, baiting and isolation techniques modified from Jung et al. (1996, 2000). Soils were sampled beneath trees of *E. gomphocephala*, *E. marginata* or *A. flexuosa* from 32 sites (four trees per site). Sites sampled included 24 sites with all stages of crown dieback, and 8 sites without visible signs of canopy decline. From each tree a total of 4 L of soil was collected from 4 points, at a distance of 50–150 cm from the stem base. Soils were sampled below the upper 5 cm organic layer to a depth of 30 cm, with attention paid to sampling along main lateral roots. The four subsamples from each tree were bulked and baited in 35 × 35 cm plastic trays. Samples were pre-moistened for 12 h before flooding with distilled water to 3–4 cm in depth above the soil line. Floating organic material was moved to the side of the baiting tray with flyscreen meshing and any remaining organic material floating on the surface of the baiting water was removed with paper towelling. Juvenile leaves of *Quercus ilex*, *Q. suber* and *Pittosporum undulatum* were floated on the water as baits. Leaves with brownish le-

sions appearing after 48–96 h were examined for the presence of *Phytophthora* sporangia using a light microscope. Leaflets with sporangia were blotted dry, and the lesions cut into 1–2 mm^2 sections and plated onto *Phytophthora* selective PARPNH medium (Jung et al. 2000). Colonies growing from the plated lesion sections were transferred to V8 agar for confirmation as *Phytophthora* isolates.

Phytophthora isolates

In addition to the five semi-papillate *Phytophthora* isolates (WAC13200–WAC13204) collected in the present study, another two isolates were used for morphological and physiological comparisons including a semi-papillate isolate from the VHS collection (DCE 236, WAC13205) previously isolated from fine roots of a recently dead *E. marginata* in the jarrah forest near Jarrahdale in 1981, and the ex-type isolate of *P. citricola* (IMI 021173) recovered from *Citrus sinensis* fruits in Taiwan (Sawada 1927) (Table 1).

Immediately prior to the present study, all isolates maintained in 90 mm Petri dishes on V8A media and as 9 mm V8A discs stored in 20 mL sterile water in McCartney bottles, were passaged through juvenile leaves of *Q. suber* used as baits on colonised agar discs flooded with sterile deionised water, and re-isolated using PARPNH selective medium.

Colony morphology, growth rates and cardinal temperatures

Hyphal morphology and colony growth patterns were described from 7 d old cultures grown at 20 °C in the dark on V8A, malt extract agar (MEA), corn-meal agar (CMA) and potato-dextrose agar (PDA) (all from BBL, Becton, Dickinson & Co, Sparks MD 21152 USA). Colony morphologies were described according to Brasier & Griffin (1979), Erwin & Ribeiro (1996) and Jung et al. (2003). Radial growth rate was recorded 5–7 d after the onset of linear growth along two lines intersecting the centre of the inoculum at right angles (Jung et al. 1999). The growth

Table 1 Isolates of *Phytophthora multivora*, *P. citricola* and *P.* '*inflata*' considered in the morphological, physiological and phylogenetic studies.

Culture no.[1]	Identification	Host	Location	Reference	GenBank Accession No.	
					ITS	*cox*1
WAC13201[2]	*P. multivora* (ex-type)	*Eucalyptus marginata*	Yalgorup, Western Australia (WA)	This study	FJ237521	FJ237508
WAC13200[2]		*E. gomphocephala*	Yalgorup, WA	This study	FJ237522	FJ237509
WAC13202[2]		*E. gomphocephala*	Yalgorup, WA	This study	FJ237520	
WAC13203[2]		*Agonis flexuosa*	Yalgorup, WA	This study	FJ237519	
WAC13204[2]		*E. gomphocephala*	Yalgorup, WA	This study	FJ237518	FJ237507
WAC13205, DCE236[2]		*E. marginata*	Jarrahdale, WA	This study	FJ237517	FJ237506
VHS16158		*Banksia menziesii*	Wanneroo, WA	This study	FJ237514	FJ237503
VHS16168		*B. grandis*	Pemberton, WA	This study	FJ237513	FJ237502
DDS1450, IMI 329674		Soil	Walpole, WA	This study	FJ237515	FJ237504
VHS16439		*B. littoralis*	Mandarah, WA	This study	FJ237516	FJ237505
P777		*E. marginata*	Western Australia	This study	FJ237525	
P15946	*P. citricola*	*Cistus canariensis*	Mallorca, Spain	Moralejo et al. (2008)	EU244846	
77a		*Quercus* sp.	Hungary	Lakatos & Szabo (unpubl.)	EU594606	
Citri-P1817			Japan	Uddin et al. (unpubl.)	AB367494	
P1817		*Medicago sativa*	South Africa	Kroon et al. (2004)		AY564170
Ps-5		*Rhododendron* sp.	Asturias, Spain	Moralejo et al. (2008)	EU194425	
83-185		*Antirrhinum majus*	Switzerland	Lefort et al. (unpubl.)	EU000083	
KACC40184		*Zizyphus jujuba*	Korea	Hong et al. (unpubl.)	AF228080	
CH455		*Mangifera indica*	Spain	Zea-Bonilla et al. (2007)	AM235209	
P16246		–	Balearic Islands, Spain	Belbahri et al. (unpubl.)	EF153674	
P142		*Rhododendron*	Switzerland	Belbahri et al. (unpubl.)	EF193230	
IF031016	*P.* '*sojae*'	–	Japan	Villa et al. (2006)	AB217685	
BR514	*Phytophthora* sp.	–	Canada	Rose et al. (unpubl.)	DQ821185	
IMI 021173, CBS 221.88[2]	*P. citricola* (ex-type)	*Citrus sinensis* fruit	Taiwan, 1927	This study	FJ237526	FJ237512
CBS 295.29	*P. citricola* (authentic)	*Citrus* leaf	Japan, 1929	This study		
CIT9		*Quercus robur*	Pulling, Germany	This study	FJ237523	FJ237510
CIT35		*Q. petraea*	Tivoli, Slovenia	This study	FJ237524	FJ237511
CIT7		*Q. robur*	Pulling, Germany	Schubert et al. (1999)	AJ007370	
MN21HH		*Rhododendron*	USA	Schwingle et al. (2007)	DQ486661	
UASWS0208		Soil from declining alder stand	Poland	Calmin et al. (unpubl.)	DQ396420	
92-198		*Taxus* sp.	Geneva, Switzerland	Belbahri et al. (unpubl.)	EF418946	
P131		*Rhododendron*	Switzerland	Belbahri et al. (unpubl.)	EF193216	
112		–	Switzerland	Bragante et al. (unpubl.)	EU263906	
Citri-P0713		–	Japan	Uddin et al. (unpubl.)	AB367492	
BR518		–	Canada?	Rose et al. (unpubl.)	DQ821180	
IMI 031372		*Rubus idaeus*	Ireland	Cooke et al. (2000)	AF266788	
6f	*P.* '*inflata*'	–	Poland	Cordier et al. (unpubl.)	EU240195	
P44		–	Slovenia	Munda et al. (unpubl.)	EF423556	
InfGaul		*Gaultheria shalon*	Scotland	Schlenzig (2005)	AY879291	AY894685
InfRhod2		*Rhododendron* sp.	Scotland	Schlenzig (2005)	AY879293	
InfVacc		*Vaccinium vitis-idaea*	Scotland	Schlenzig (2005)	AY879292	AY894684
804		Soil from declining alder stand	Poland	Cordier et al. (unpubl.)	EU240058	
IMI 342898		*Syringa vulgaris*	UK	Cooke et al. (2000) – ITS Kroon et al. (2004) – *cox*1	AF266789	AY564187

[1] Abbreviations of isolates and culture collections: CBS = Centraalbureau voor Schimmelcultures Utrecht, Netherlands; IMI = CABI Bioscience (Imperial Mycological Institute), UK; WAC = Department of Agriculture and Food Western Australia Plant Pathogen Collection, Perth, Australia; VHS = Vegetation Health Service of the Department of Environment and Conservation, Perth, Australia; DDS, DCE = earlier prefixes of VHS collection. Other isolate names and numbers are as given on GenBank.

[2] Isolates used in the morphological and growth-temperature studies.

test was repeated once. For temperature growth studies, all isolates were subcultured onto V8A plates and incubated for 24 h at 20 °C to initiate growth. Three replicate plates for each isolate and temperature were then transferred to incubators set at 10, 15, 17.5, 20, 22.5, 25, 30 and 32.5 °C, and radial colony growth was measured as above after 5–7 d.

Morphology of sporangia and gametangia

Sporangia and gametangia were produced on V8A and measurements were made as described by Jung et al. (1999). Sporangia were obtained by flooding 5 × 5 mm square agar discs taken from growing margins of 3–5 d old colonies with non-sterile soil extract in 90 mm Petri dishes and incubating them in the dark at 18–22 °C for 12–16 h. The non-sterile soil extract was obtained by flooding 100 mL of commercial composted potting mix (Richgro, Jandakot, WA) with 1 L of deionised water. After 24 h at 10–25 °C, the soil extract was removed from the water surface with a pipette and diluted to 10 % with deionised water. Dimensions and characteristic features of 50 mature sporangia chosen at random were determined at ×400 magnification (BH-Olympus) for each isolate. For each isolate dimensions and characteristic features of 50 mature oogonia, oospores and antheridia, and diameters of 25 primary hyphae chosen at random were measured at ×400 magnification at the surface of 15 mm discs cut from the centre of 14–22 d old V8A cultures grown in the dark at 20 °C. For each isolate the oospore wall index was calculated as the ratio between the volume of the oospore wall and the volume of the entire oospore (Dick 1990).

DNA isolation, amplification and sequencing

The *Phytophthora* isolates were grown on half strength PDA (Becton, Dickinson and Company, Sparks, USA, 19.5g PDA, 7.5g of agar and 1L of distilled water) at 20 °C for 2 wk and the mycelium was harvested by scraping from the agar surface with a sterile blade and placed in a 1.5 mL sterile Eppendorf® tube. Harvested mycelium was frozen in liquid nitrogen, ground to a fine powder and genomic DNA was extracted according to Andjic et al. (2007). The region spanning the internal transcribed spacer (ITS)1-5.8S-ITS2 region of the ribosomal DNA was amplified using the primers ITS-6 (5' GAA GGT GAA GTC GTA ACA AGG 3') (Cooke et al. 2000) and ITS-4 (5'TCC TCC GCT TAT TGA TAT GC 3') (White et al. 1990). The PCR reaction mixture, PCR conditions, the clean-up of products and sequencing were as described by Andjic et al. (2007).

The mitochondrial gene *cox*1 was amplified with primers Fm84 (5'TTT AAT TTT TAG TGC TTT TGC) and Fm83 (5'CTC CAA TAA AAA ATA ACC AAA AAT G) (Martin & Tooley 2003). The PCR reaction mixture was the same as for the ITS region, but the PCR conditions were as described previously (Martin & Tooley 2003). Templates were sequenced in both directions with primers used in amplification, as well as primers FM 85 (5'AAC TTG ACT AAT AAT ACC AAA) and FM 50 (5'GTT TAC TGT TGG TTT AGA TG) (Martin & Tooley 2003). The clean-up of products and sequencing were the same as for the ITS region.

Phylogenetic analysis

In order to compare *Phytophthora* isolates used in this study with other closely related species (ITS clade 2, Cooke et al. 2000), additional sequences were obtained from GenBank (Table 1). Sequences were also obtained for species representing other ITS clades (Cooke et al. 2000). Sequence data for the ITS region were initially assembled using Sequence Navigator v. 1.01 (Perkin Elmer) and aligned in Clustal X (Thompson et al. 1997). Manual adjustments were made visually by inserting gaps where necessary in BioEdit v. 5.0.6 (Hall 2001). There were no gaps in the *cox*1 alignment. All sequences derived in this study were deposited in GenBank and accession numbers are shown in Table 1.

Parsimony analysis was performed in PAUP (Phylogenetic Analysis Using Parsimony) v. 4.0b10 (Swofford 2003). The most parsimonious trees were obtained using heuristic searches with random stepwise addition in 100 replicates, with the tree bisection-reconnection branch-swapping option on and the steepest-descent option off. Maxtrees were unlimited, branches of zero length were collapsed and all multiple, equally parsimonious trees were saved. Estimated levels of homoplasy and phylogenetic signal (retention and consistency indices) were determined (Hillis 1992). Branch and branch node support was determined using 1 000 bootstrap replicates (Felsenstein 1985).

Bayesian analysis was conducted on the same individual dataset as that used in the parsimony analysis. First, MrModeltest v. 2.5 (Nylander J.A.A. 2004. Program distributed by the author. Evolutionary Biology Centre, Uppsala University) was used to determine the best nucleotide substitution model. Phylogenetic analyses were performed with MrBayes v. 3.1 (Ronquist & Huelsenbeck 2003) applying a general time reversible (GTR) substitution model with gamma (G) and proportion of invariable site (I) parameters to accommodate variable rates across sites. Two independent runs of Markov Chain Monte Carlo (MCMC) using 4 chains were run over 1 000 000 generations. Trees were saved each 1 000 generations, resulting in 10 001 trees. Burn-in was set at 101 000 generations (i.e. 101 trees), well after the likelihood values converged to stationary, leaving 9 900 trees from which the consensus trees and posterior probabilities were calculated.

Statistical analysis

Analyses of Variances were carried out using Statistica v. 5.1 (Statsoft Inc., Tulsa, Oklahoma) to determine whether physiological and morphological measurements were different between isolates.

RESULTS

Phylogenetic analysis

The ITS dataset consisted of 894 characters of which 420 were parsimony informative. The dataset contained significant phylogenetic signal compared to 1 000 random trees ($p < 0.01$, $g1 = -1.38$). Heuristic searches resulted in 10 most parsimonious trees of 848 steps (CI = 0.72, RI = 0.89). The topology of the Bayesian tree was very similar (TreeBASE SN4153) (Fig. 2). Several ITS sequences from GenBank are identical to *P. multivora* (AB217685, AB367494, AF228080, AM235209, DQ821185, EF153674, EF193230, EU000083, EU194425, EU244846, EU594606). These sequences were not all included in the phylogenetic analysis, but information on origin and studies they have derived from is given in Table 1 and the position of polymorphic nucleotides indicating their similarity to *P. multivora* in Table 2. In addition, on GenBank there are several isolates of *P. multivora*, originally designated as *P*. sp. 4 by Burgess et al. (In press) (EU301126–32 and EU0869194–99).

All isolates of *P. multivora* reside in a strongly supported terminal clade clearly distinct from the ex-type and authentic type of *P. citricola* (IMI 021173 and CBS 295.29) within ITS clade 2 (Cooke et al. 2000). Two additional isolates listed on GenBank (Citri-P0713 and BR518) have identical sequence to the ex-type of *P. citricola* (IMI 021173) (Fig. 2). There are seven fixed polymorphisms that are different between *P. multivora* and IMI 021173 (Table 2). Isolates listed on GenBank as *P. citricola* from the Northern Hemisphere (CIT7, CIT9, CIT35, P44, MN21HH, UASWS0208, 92-198, P131, IMI031372, 112) differ by at least 10 bp from *P. multivora*. (Fig. 2, Table 2). Isolates listed on

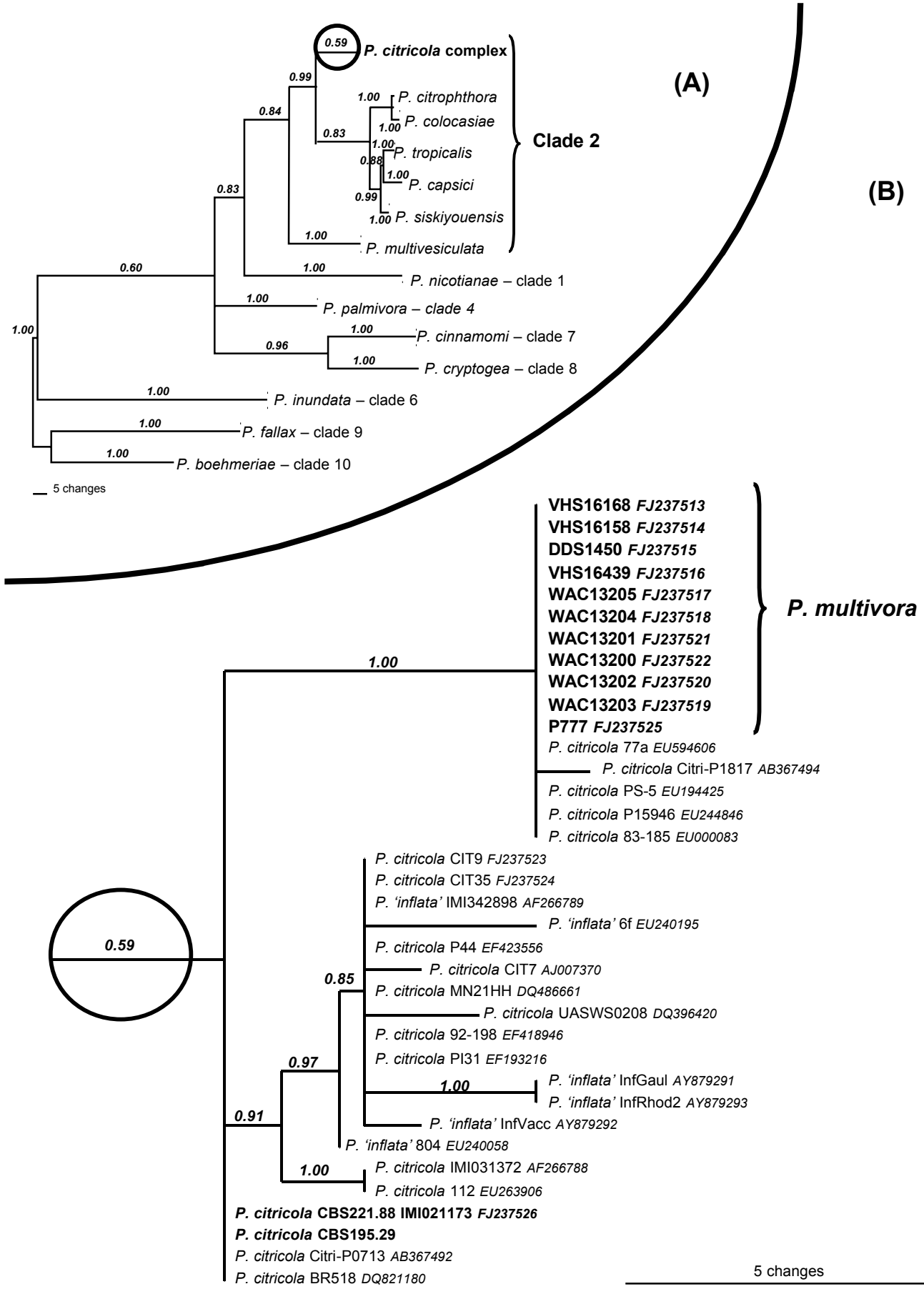

Fig. 2 Bayesian inference tree using rDNA ITS sequences showing phylogenetic relationships between (A) clade 2 species and representative species from other clades and (B) isolates from the *P. citricola* complex. Numbers above branches represent posterior probability based on Bayesian analysis of the dataset. Both trees result from a single analysis as given in TreeBASE (SN4153). For tree A, clades were collapsed to show the relationship between isolates from *P. citricola* complex and other species in clade 2. Tree B shows the finer details within the *P. citricola* complex (node enclosed in circle on tree A) and the relationship between *P. multivora* and other *P. citricola* and *P. 'inflata'* isolates including the ex-type of *P. citricola* (IMI 021173).

Table 2 Positions of polymorphic nucleotides (bp) from aligned sequence data of the ITS gene region showing the variation between *Phytophthora multivora*, *P. citricola* and *P.* '*inflata*' isolates. Polymorphisms that differ from the type of *P. multivora* (WAC13201) are in blue.

Isolate no.	4	15	20	43	54	67	154	397	412	485	543	633	650	704	736
	P. multivora														
WAC13201	A	A	C	C	C	–	T	T	A	C	G	A	G	C	T
VHS16439	A	A	C	C	C	–	T	T	A	C	G	A	G	C	T
WAC13205	A	A	C	C	C	–	T	T	A	C	G	A	G	C	T
WAC13204	A	A	C	C	C	–	T	T	A	C	G	A	G	C	T
WAC13203	A	A	C	C	C	–	T	T	A	C	G	A	G	C	T
WAC13202	A	A	C	C	C	–	T	T	A	C	G	A	G	C	T
WAC13200	A	A	C	C	C	–	T	T	A	C	G	A	G	C	T
P777	A	A	C	C	C	–	T	T	A	C	G	A	G	C	T
VHS16168	A	–	C	C	C	–	T	T	A	C	G	A	G	C	T
VHS16158	A	–	C	C	C	–	T	T	A	C	G	A	G	C	T
DDS1450	A	–	C	C	C	–	T	T	A	C	G	A	G	C	T
P15946	A	–	C	C	C	–	T	T	A	C	G	A	G	C	T
PS-5	A	–	C	C	C	–	T	T	A	C	G	A	G	C	T
77a	A	–	C	C	C	–	T	T	A	C	G	A	G	C	T
Citri-P1817	A	–	C	C	C	–	T	T	A	C	G	A	G	C	T
83-185	A	–	C	C	C	–	T	T	A	C	G	A	G	C	T
KACC40184	A	–	C	C	C	–	T	T	A	C	G	A	G	C	T
IF031016	A	–	C	C	C	–	T	T	A	C	G	A	G	C	T
BR514	A	–	C	C	C	–	T	T	A	C	G	A	G	C	T
CH455	A	–	C	C	C	–	T	T	A	C	G	A	G	C	T
P16246	A	–	C	C	C	–	T	T	A	C	G	A	G	C	T
P142	A	–	C	C	C	–	T	T	A	C	G	A	G	C	T
	P. citricola														
IMI 021773*	A	–	C	T	T	T	T	T	G	T	G	G	G	T	T
CBS 295.29	A	–	C	T	T	T	T	T	G	T	G	G	G	T	T
Citri-P0713	A	–	C	T	T	T	T	T	G	T	G	G	G	T	T
BR518	A	–	C	T	T	T	T	T	G	T	G	G	G	T	T
CIT7	A	–	T	T	T	T	C	C	G	T	G	G	G	T	T
CIT9	A	–	T	T	T	T	C	C	G	T	G	G	G	T	T
CIT35	A	–	T	T	T	T	C	C	G	T	G	G	G	T	T
P44	A	–	T	T	T	T	C	C	G	T	G	G	G	T	T
MN21HH	A	–	T	T	T	T	C	C	G	T	G	G	G	T	T
UASWS0208	A	–	T	T	T	T	C	C	G	T	G	G	G	T	T
92-198	A	–	T	T	T	T	C	C	G	T	G	G	G	T	T
P131	A	–	T	T	T	T	C	C	G	T	G	G	G	T	T
IMI 031372	A	–	T	T	T	–	T	T	G	T	A	G	A	T	T
112	A	–	T	T	T	–	T	T	G	T	A	G	A	T	T
	***P.* '*inflata*'**														
IMI 342898	A	–	T	T	T	T	C	C	G	T	G	G	G	T	T
804	A	–	T	T	T	T	C	T	G	T	G	G	G	T	T
6f	A	–	T	T	T	T	C	C	G	T	G	G	G	T	T
Infgaul	C	–	T	T	T	T	C	C	G	T	G	G	G	T	A
InfRhod2	C	–	T	T	T	T	C	C	G	T	G	G	G	T	A
InfVacc	A	–	T	T	T	T	C	C	G	T	G	G	G	T	T

GenBank as *P. 'inflata'* (6f, P44, InfGaul, InfRhod2, InfVacc, 804, IMI 342898) are dispersed among the northern hemisphere isolates of *P. citricola* (Fig. 2, Table 2) and it is unclear whether any of these isolates represent the original *P. inflata.*

The *cox*1 dataset consisted of 742 characters of which 107 were parsimony informative. The dataset contained significant phylogenetic signal compared to 1 000 random trees ($p < 0.01$, $g1 = -0.66$). Heuristic searches resulted in 12 most parsimonious trees of 301 steps (CI = 0.50, RI = 0.67). The topology of the Bayesian tree was very similar (TreeBASE SN4153) (Fig. 3). Species from ITS clade 2 group together with strong support. Isolates of *P. multivora* were in a separate clade from the ex-type isolate of *P. citricola* (IMI 021173) and two European isolates (CIT9 and CIT35) also sequenced in this study, and the isolates listed on GenBank as *P. inflata.* There was another single sequence of *P. citricola*, P1817, available from the study of Kroon et al. (2004). This sequence was distinct from our sequences for *P. citricola* and *P. multivora*, all GenBank sequences for *P. inflata* and other ITS clade 2 species, and, based on the findings of this study, must be either an incorrectly identified isolate or an incorrect sequence.

Taxonomy

Phytophthora multivora P.M. Scott & T. Jung, *sp. nov.* — MycoBank MB512497; Fig. 4, 5

Sporangia abundantia in cultura liquida, persistentia, terminalia, semi-papillata, ovoidea aut limoniformia, rare distorta vel bipapillata, 53 ± 10.1 × 31.8 ± 6.2 µm, ratio longitudo ad altitudinem 1.7 ± 0.2 µm. Systema sexus homothallica; oogonia globosa vel rare subglobosa, 27.1 ± 2.1 µm. Oosporae fere pleroticae, 23.9 ± 2 µm, paries 2.6 ± 0.5 µm. Antheridia paragynosa, 13 ± 2.2 × 8.8 ± 1.1 µm. Chlamydosporae et inflationes hypharum non observatae. Temperaturae crescentiae in agaro 'V8A', optima c. 25 °C et maxima 30–32.5 °C. Coloniae in agaro 'V8A' stellatae cum mycelio aerio restricto et margine submersa. Regiones 'rDNA ITS' et '*cox*1' cum unica sequentia (GenBank FJ237508, FJ237521).

Etymology. Name refers to the wide host range (multi L. = many, -vora L. = feeding).

Sporangia (Fig. 4) — Sporangia were rarely observed on solid agar but were produced abundantly in non-sterile soil extract. The majority of sporangia for all *P. multivora* isolates and the ex-type of *P. citricola* (IMI 021173) were formed between 7–12 h after flooding with soil extract. Little variation in sporangial shapes was observed between the *P. multivora* isolates. The majority of sporangia were semipapillate and either ovoid, limoniform, ellipsoid or obpyriform (Fig. 4a–d, g–i), sometimes with just a very shallow apical thickening (Fig. 4f), non-cadu-

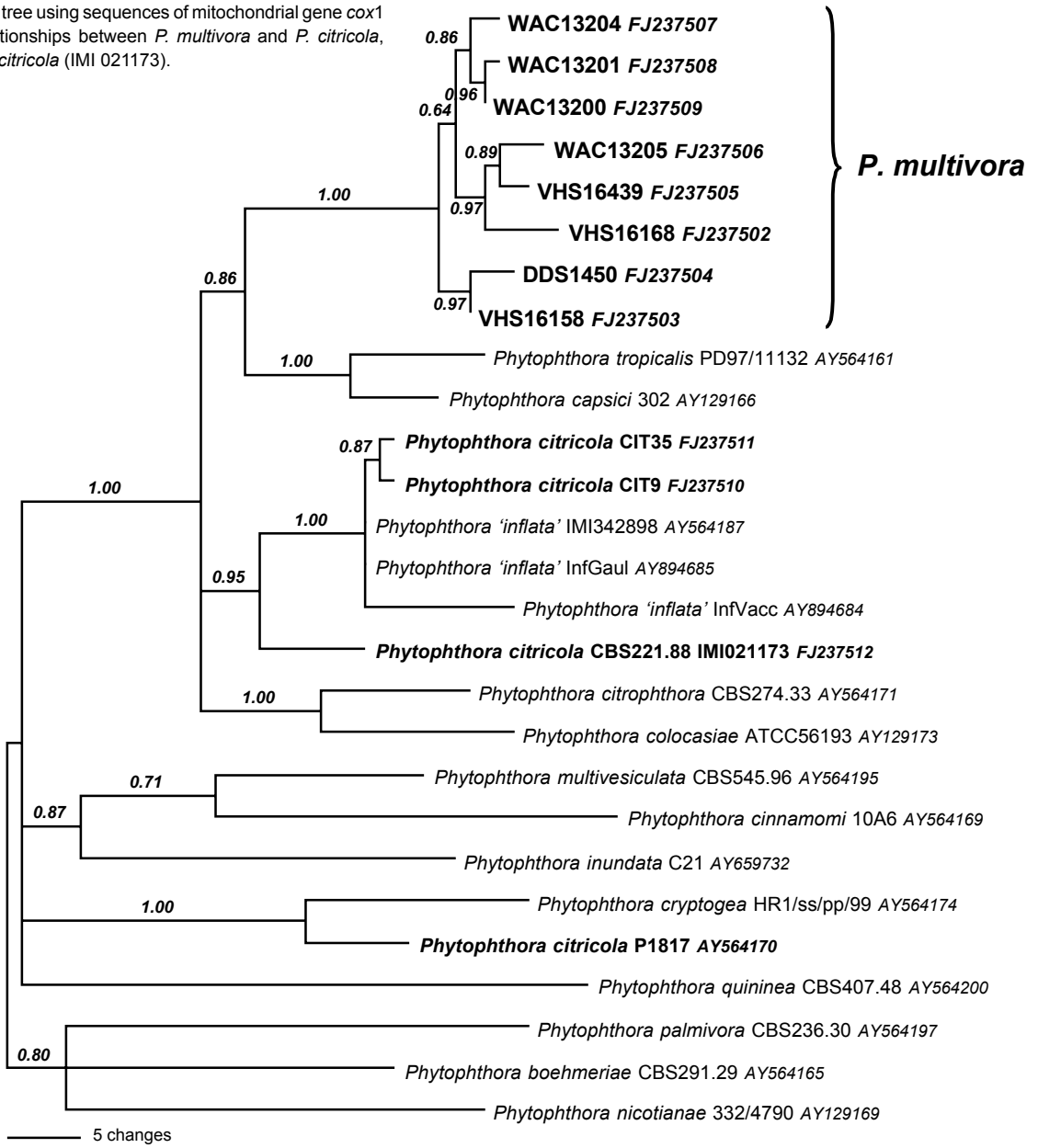

Fig. 3 Bayesian inference tree using sequences of mitochondrial gene *cox*1 showing phylogenetic relationships between *P. multivora* and *P. citricola*, including the ex-type of *P. citricola* (IMI 021173).

cous, occasionally forming a conspicuous basal plug (Fig. 4i) that protruded into the empty sporangium. Sporangia with two or three papillae or distorted shapes were occasionally formed by all isolates (Fig. 4e, f, j–l). Sporangia were typically borne terminally (Fig. 4a–f, j–l) but some were laterally attached (Fig. 4g) or intercalary (Fig. 4i). External proliferation was regularly observed (Fig. 4a–d, j, l), either irregular or in lax or dense sympodia. The majority of sporangia of each isolate had released zoospores between 15–20 h after flooding. Compared to *P. citricola*, sporangia of *P. multivora* showed a higher proportion of abortion or direct germination (Fig. 4j–l) after 24–48 h within the same soil extract. After 24–48 h, bell-shaped sporangia were formed by all six isolates of *P. multivora* which germinated directly from two points without prior formation of papillae (Fig. 4k, l). The mean sporangial dimensions of the six *P. multivora* isolates were 51.0 ± 10.4 × 30.0 ± 5.1 µm (overall range of 25–97 × 13–63 µm) with a length/breadth ratio of 1.7 ± 0.22 (overall range 1.3–3.3). The mean sporangial dimensions of the ex-type of *P. citricola* (IMI 021173), at 50.9 ± 6.9 × 29.9 ± 5.1 µm (range 39–70 × 22–40 µm) and a length/breadth ratio of 1.7 ± 0.3 (overall range 1.3–2.6), were within the range of the *P. multivora* isolates (Table 3). In contrast to *P. multivora*, sporangia of *P. citricola* were generally more variable and often showed distorted shapes including: multiple papillae, curved apices and hyphal beaks. Twelve percent of sporangia of *P. citricola* were distorted compared to 5 % in *P. multivora*. With 9 % and 10 %, respectively, *P. multivora* and *P. citricola* had a similar proportion of sporangia with lateral attachment to the sporangiophore. Isolate WAC13204 was different from all other *P. multivora* isolates by forming significantly ($p < 0.05$) larger sporangia with a mean size of 62.3 ± 10.8 × 34.0 ± 4.9 µm.

Oogonia, oospores and antheridia (Fig. 5a–e) — Gametangia were readily produced in single culture by all *P. multivora* isolates and the ex-type of *P. citricola* (IMI 021173) on V8A within 4 d. Oogonia of both *P. multivora* and *P. citricola* were borne terminally, had smooth walls and were globose to slightly subglobose (Fig. 5a–d). With a mean diam of 26.5 ± 1.9 µm (overall range 19–37 µm and range of isolate means 25.5–27.8 µm) the oogonia of the six *P. multivora* isolates were on average smaller than those of *P. citricola* (30.3 ± 2.7 µm, range 22–34 µm), although the ranges were broadly overlapping (Table 3). Oospores of both *P. multivora* (Fig. 5b–d) and *P. citricola* were globose and nearly plerotic. The *P. multivora* isolates produced significantly ($p < 0.05$) thicker oospore walls (2.6 ± 0.5 µm, overall range 1.4–4.6 µm) than *P. citricola* (1.9 ± 0.3 µm, overall range 1.2–2.6). Due to the smaller oospore size

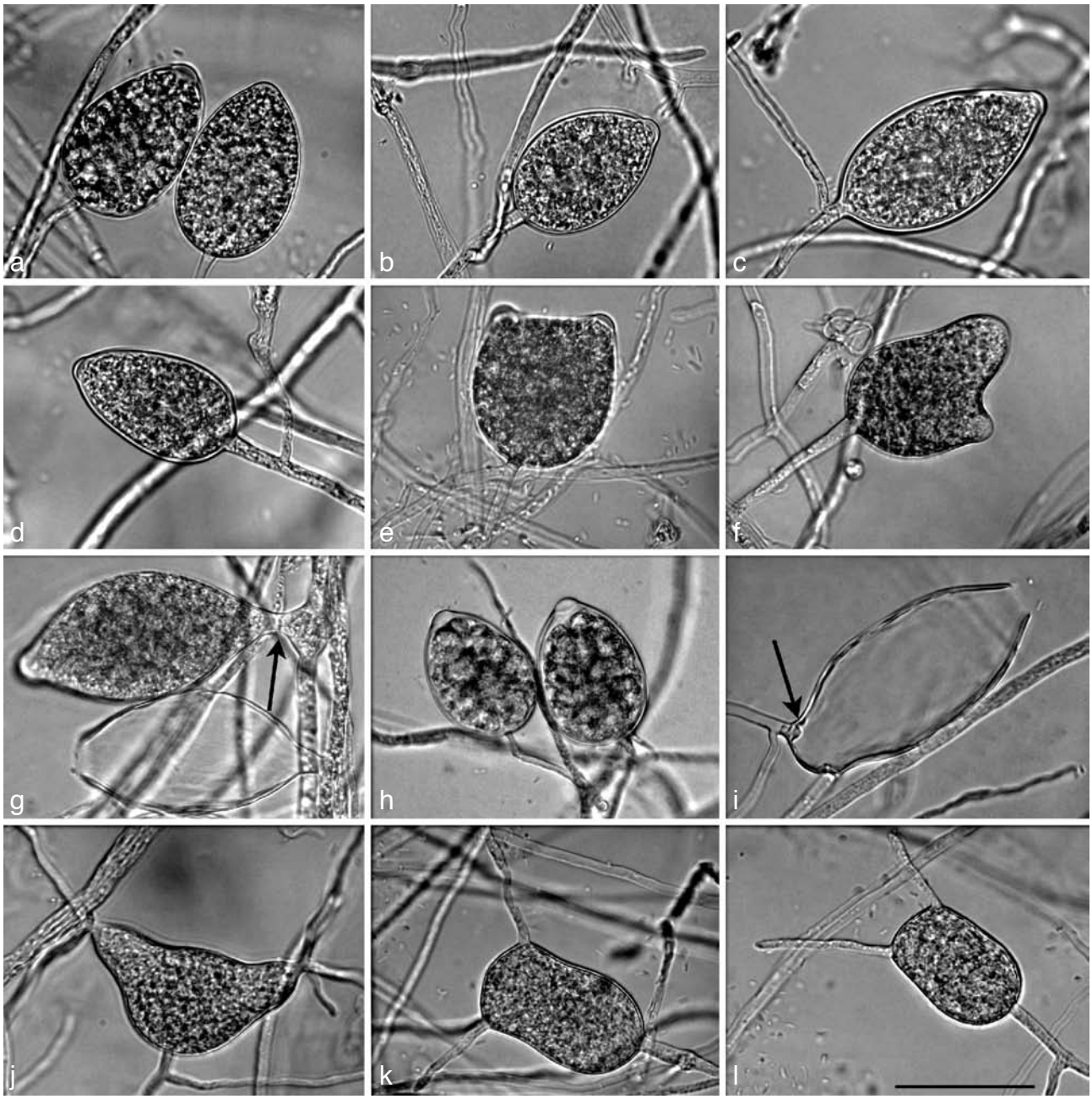

Fig. 4 Semipapillate sporangia of *Phytophthora multivora* on V8 agar. — a–i after 12–24 h flooding with soil extract. a. Ovoid, the left sporangium with swollen papilla shortly before release of the already differentiated zoospores; b. ovoid; c. limoniform; d. obpyriform; e. bipapillate; f. bipapillate, bilobed; g. limoniform, laterally inserted to the sporangiophore (arrow); h. ovoid, shortly before release of zoospores; i. limoniform, intercalary inserted, with conspicuous basal plug (arrow) protruding into empty sporangium after release of zoospores. — j–l direct germination after 48 h flooding. j. Bipapillate, bilobed with several germ tubes growing from each papilla; k, l. bipapillate, bell-shaped with one germ tube growing from each papilla. — Scale bar = 50 µm, applies to a–l.

of *P. multivora* the oospore wall index was significantly higher ($p < 0.0001$) in *P. multivora* (0.52 ± 0.07) than in *P. citricola* (0.36 ± 0.05). Antheridia of both species were obovoid, club-shaped or irregular, almost exclusively paragynous, diclinous and typically attached close to the oogonial stalk. Intercalary and amphigynous antheridia were only rarely observed. After 4 wk in V8A at 20 °C, more than 90 % of all *P. multivora* oospores had germinated directly. Since the thick inner oospore wall of *Phytophthora* species erodes during the germination process due to enzymatic digestion of its major components, the glucans, (Erwin & Ribeiro 1996) only the thin outer oospore wall surrounded by the thin oogonial wall was left (Fig. 5e). No direct germination was observed in cultures of the ex-type of *P. citricola* (IMI 021173) growing under the same conditions.

Colony morphology, growth rates and cardinal temperatures — Colony growth patterns of two isolates of *P. multivora* (WAC13201 and WAC13205) and the ex-type isolate of *P. citricola* (IMI 021173) are shown in Fig. 6. All *P. multivora* isolates except isolate WAC13204 produced similar colony growth patterns on the four different types of media. On V8A, CMA and MEA *P. multivora* isolates produced limited aerial mycelium and distinct growth patterns, while isolate WAC13204 formed fluffy to felty, uniform colonies without distinct growth pattern. The colony morphology on V8A and MEA of all *P. multivora* isolates clearly differed from the colony morphology of the ex-type isolate of *P. citricola* (IMI 021173). *Phytophthora multivora* isolates produced stellate growth patterns with a clearly delimited, submerged margin on V8A and faintly stellate to dendroid patterns on MEA while *P. citricola* formed a typical chrysanthemum pattern on both media. On CMA, *P. multivora* isolates formed appressed to submerged colonies with a faintly stellate to petaloid pattern while *P. citricola* produced even sparser submerged colonies with a faintly stellate pattern. On PDA, the *P. multivora* isolates and *P. citricola* produced petaloid

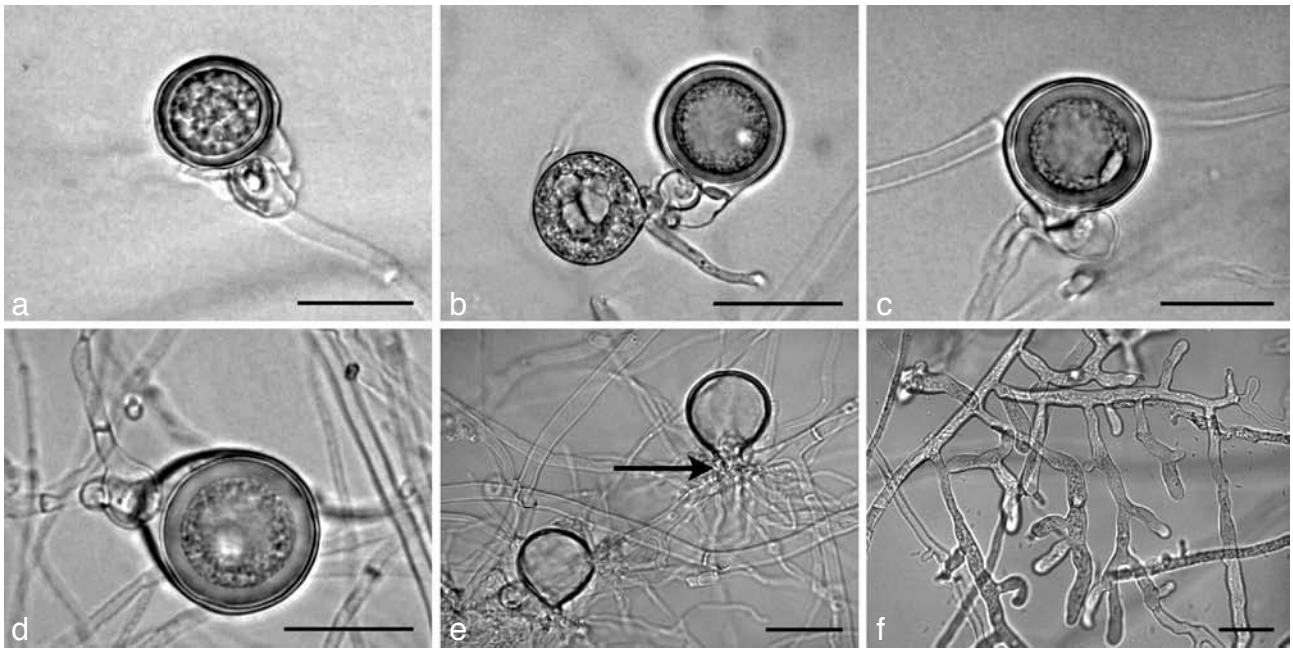

Fig. 5 a–d. Oogonia of *Phytophthora multivora* with paragynous antheridia and plerotic oospores on V8 agar. a. Juvenile oogonium with thin-walled oospore and undifferentiated cytoplasm; b–d. mature oogonia with thick-walled oospores and ooplast; b. oogonium on the left side is aborted; e. direct germination of oospores with several germtubes through the oogonial bases (arrow) after 5 wk incubation at 20 °C; f. tubular, irregular lateral hyphae. — Scale bars = 25 µm.

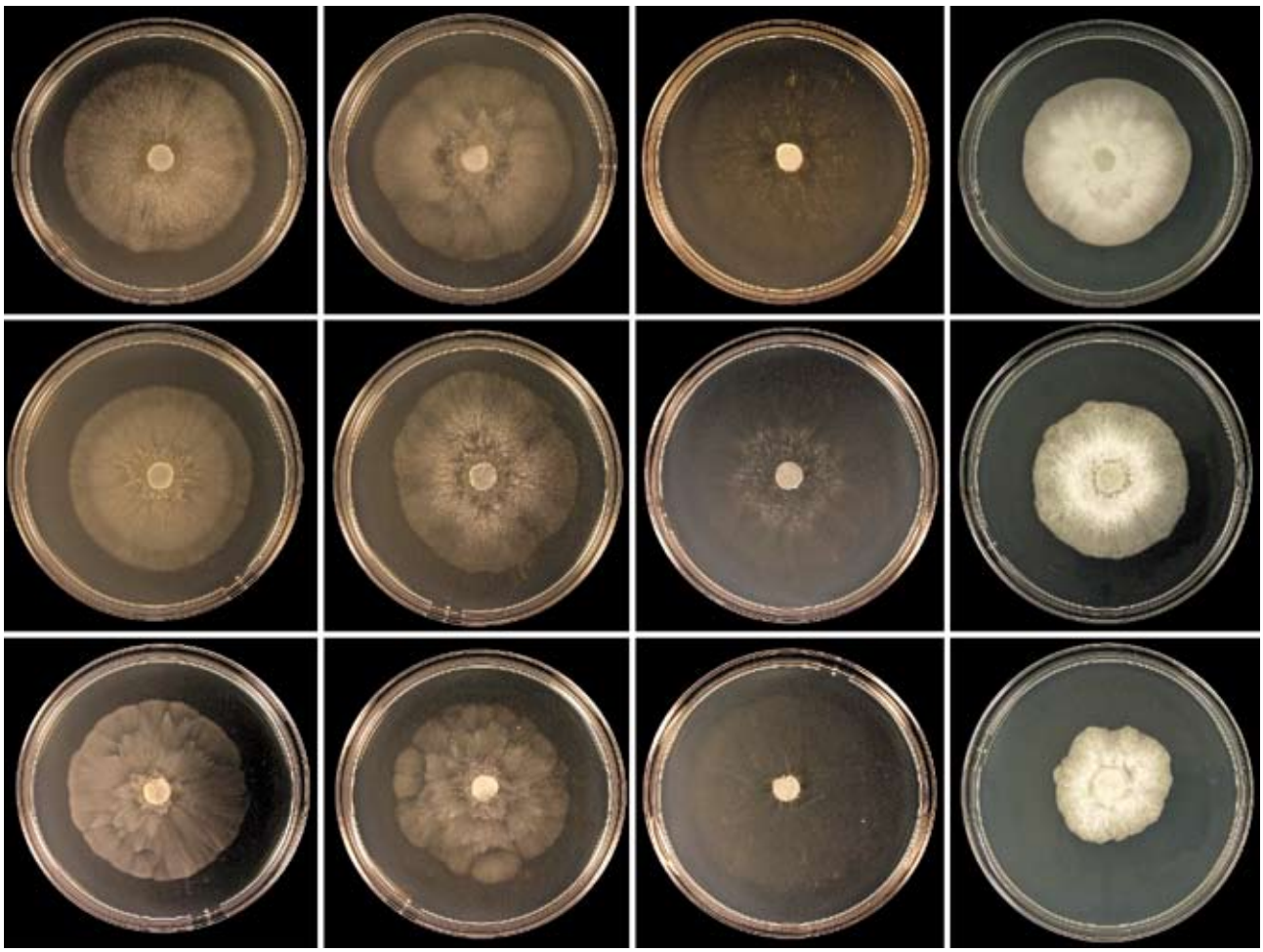

Fig. 6 Colony morphology of isolates WAC13201 (ex-type) and WAC13205 of *Phytophthora multivora*, and the ex-type isolate of *P. citricola* (from top to bottom) after 6 d growth at 20 °C on V8 agar, malt extract agar, cornmeal agar and potato-dextrose agar (from left to right).

Table 3 Morphological dimensions (µm) and temperature-growth relations of *Phytophthora multivora* and *P. citricola*.

	P. multivora						*P. citricola*
Isolate no.[1]	WAC13200	WAC13201[2]	WAC13202	WAC13203	WAC13204	WAC13205	IMI 021173[2]
Sporangia							
L×b mean	56.5 ± 7 × 31.8 ± 4	53 ± 10.1 × 31.8 ± 6.2	44.2 ± 4.4 × 26.2 ± 3.1	44.5 ± 7.8 × 28.9 ± 4.2	62.3 ± 10.8 × 34.0 ± 4.9	45.7 ± 5.2 × 27.9 ± 3.6	50.9 ± 6.9 × 29.9 ± 5.1
Range	38–72 × 25–41	38–97 × 24–63	36–58 × 13–33	33–65 × 24–45	25–86 × 18–44	37–58 × 20–34	39–70 × 22–40
l/b ratio	1.8 ± 0.2	1.7 ± 0.2	1.7 ± 0.2	1.5 ± 0.1	1.8 ± 0.3	1.6 ± 0.1	1.7 ± 0.3
Oogonia							
Mean diam	25.6 ± 1.3	27.1 ± 2.1	25.5 ± 1.4	26.2 ± 1.5	26.8 ± 1.5	27.8 ± 2.5	30.3 ± 2.7
diam range	23–28	23–31	21–28	21–29	23–30	19–37	22–34
Oospore							
mean diam	22.7 ± 1.3	23.9 ± 2	22.8 ± 1.4	23.3 ± 1.5	23.8 ± 1.7	24.8 ± 2.2	27.3 ± 2.6
diam range	20–26	19–28	18–26	19–26	21–28	17–31	20–30
wall diam	2.6 ± 0.4	2.6 ± 0.5	2.7 ± 0.4	2.7 ± 0.3	2.3 ± 0.4	2.5 ± 0.5	1.9 ± 0.3
Antheridia							
L×b mean	13.1 ± 1.8 × 8.8 ± 1.3	13 ± 2.2 × 8.8 ± 1.1	11.7 ± 1.9 × 7.9 ± 1	13.5 ± 1.6 × 9 ± 1.4	12.4 ± 1.6 × 8.7 ± 1.4	13.8 ± 1.8 × 8.5 ± 1.2	13.2 ± 2.5 × 8.1 ± 1.8
L×b range	9–17 × 6–13	8–18 × 7–12	8–16 × 5–10	10–16 × 6–14	9–17 × 5–12	10–20 × 6–11	7–19 × 5–14
Maximum temperature (°C)	30–32.5	30–32.5	30–32.5	30–32.5	> 32.5	30–32.5	30–32.5
Optimum temperature (°C)	25	25	25	25	25	25	22.5
Growth rate on V8A at optimum (mm/d)	4.7	5.7	5.7	5.7	6.1	5.4	5.7
Growth rate at 20 °C (mm/d)							
V8A	4.0	4.0	4.4	4.5	4.7	3.8	5.2
MEA	4.1	3.8	3.4	3.6	4.7	3.4	4.0
CMA	4.4	4.5	3.3	3.3	4.5	3.5	4.8
PDA	3.1	2.9	2.6	2.5	3.7	2.9	2.3

[1] For isolate details see Table 1. [2] Ex-type isolate.

felty to fluffy colonies. Diameters of primary hyphae varied from 3.8–4.6 µm. Lateral hyphae of *P. multivora* were often tubular and slightly inflated (Fig. 5f). No substantial differences were observed between hyphae of *P. multivora* and *P. citricola*.

Temperature growth relations of *P. multivora* and the ex-type isolate of *P. citricola* are shown in Fig. 7. The maximum growth temperature for isolates of both *P. multivora* and the ex-type of *P. citricola* (IMI 021173) on V8A was between 30–32.5 °C. All isolates of *P. multivora* except isolate WAC13204 were unable to grow at 32.5 °C, but started re-growth within 12 h when plates that were incubated for 7 d at 32.5 °C were transferred to 25 °C. All six *P. multivora* isolates had a growth optimum at 25 °C with growth rates ranging from 4.7–6.1 mm/d while *P. citricola* showed a broad growth optimum between 22.5 °C (5.7 mm/d) and 30 °C (5.5 mm/d). Compared to all *P. multivora* isolates the growth rate of *P. citricola* at 20 °C was higher on V8A and CMA and lower on PDA (Table 2). On V8A, over the whole temperature range except at 25 °C, all *P. multivora* isolates were markedly slower growing than *P. citricola* (Fig. 7).

Fig. 7 Radial growth rates of *Phytophthora multivora* (means and standard errors calculated from six isolates), solid line, and the ex-type isolate of *P. citricola*, dashed line, on V8 agar at different temperatures.

Specimens examined. Western Australia, Yalgorup, from rhizosphere soil of declining *Eucalyptus marginata*, May 2007, *P. Scott* & *T. Jung*, holotype MURU 434 (dried culture on V8A, Herbarium of Murdoch University, Western Australia), culture ex-type WAC13201; from rhizosphere soil of declining *Eucalyptus gomphocephala*, May 2007, *T. Jung* & *P. Scott*, WAC13200; from rhizosphere soil of declining *Eucalyptus gomphocephala*, June 2007, *P. Scott*, WAC13202; from rhizosphere soil of declining *Agonis flexuosa*, June 2007, *P. Scott*, WAC13203; from rhizosphere soil of declining *Eucalyptus gomphocephala*, June 2007, *P. Scott*, WAC13204; North Jarrahdale, from rhizosphere soil of declining *Eucalyptus marginata*, 1980, *unknown*, WAC13205.

Notes — In previous studies *P. multivora* is referred to as *P. citricola* (Shearer et al. 1987, 1988, Shearer & Tippett 1989, Bunny 1995, Stukely et al. 1997), and more recently as *P.* sp. 4 (Burgess et al. In press). Many isolates from a wide range of host species in WA that had been identified as *P. citricola* in the past must be reassigned to *P. multivora*. As indicated above, *P. multivora* has been isolated from the south-west of WA from rhizosphere soil of *E. gomphocephala*, *E. marginata* and *A. flexuosa* in Yalgorup National Park. It has also been recovered by the VHS from soil and root samples collected beneath dying, *Phytophthora*-sensitive 'indicator species' in native ecosystems in the south-west of WA by the VHS over the last 30 yr, which extends the host list to include *Banksia attenuata*, *B. grandis*, *B. littoralis*, *B. menziesii*, *B. prionotes*, *Conospermum* sp., *Leucopogon verticillatus*, *Xanthorrhoea gracilis*, *Podocarpus drouyniana*, *Patersonia* sp., *Bossiaea* sp., *Gastrolobium spinosum* and *Pinus radiata* (plantation) (Burgess et al. In press). *Phytophthora multivora* has also recently been isolated from large girdling stem lesions of *B. attenuata* in Injidup, WA (G. Hardy unpubl. data, Fig. 1e, f), and from fine roots of

declining *E. marginata* in the Jarrah forest near Jarrahdale in 1981 (M. Stukely unpubl. data) and near Dwellingup in 2008 (T. Jung unpubl. data).

DISCUSSION

Phytophthora multivora was previously identified as *P. citricola* in WA based solely on morphological characters including homothallic breeding behaviour, production of paragynous antheridia, semipapillate persistent sporangia and oogonia with dimensions in the correct range, absence of catenulate hyphal swellings in liquid culture, and similar growth rates at 25 °C. Phylogenetic analyses of the ITS and *cox*1 gene regions show that *P. multivora* is unique and comprises a discrete cluster within the major ITS clade 2 of Cooke et al. (2000) with its present closest relative being *P. citricola*.

Morphological and molecular studies using a broad range of *P. citricola* isolates have demonstrated that *P. citricola* is very diverse (Oudemans et al. 1994, Bhat & Browne 2007, Moralejo et al. 2008), and that many of the differences are associated with host and geography (Oudemans et al. 1994, Bhat & Browne 2007). In the isozyme study of Oudemans et al. (1994) a global collection of 125 isolates of *P. citricola* clustered into five distinct subgroups suggesting *P. citricola* is a species complex instead of a single species which is to be expected considering the broad geographic and host range of *P. citricola* isolates (Fontaneto et al. 2008).

Even though multiple *P. citricola* sequences have been submitted to GenBank, sequence data for the ex-type of *P. citricola* (IMI 021173) from *Citrus sinensis* fruits in Taiwan (Sawada 1927) has not previously been available, and this has led to confusion in the phylogeny. Besides the ex-type culture, an authentic type of *P. citricola* (CBS 295.29) isolated from *Citrus* leaves in Japan was submitted to CBS in 1929 by Sawada. The present study is the first to provide sequence data of these isolates, and our results clearly demonstrate that the isolates from WA constitute a new species, *P. multivora*. Isolates designated as *P. 'inflata'* were distributed through the *P. citricola* complex demonstrating the difficulty in distinguishing between *P. inflata* and *P. citricola*. The original *P. inflata* ex-type from elm trees in the United States (Caroselli & Tucker 1949) has been lost and it has been suggested that designated isolates of *P. inflata* from other hosts (Hall et al. 1992) are conspecific with *P. citricola* (Cooke et al. 2000). Among isolates from the *P. citricola* complex, isolates now described as *P. multivora* are the most distant to the ex-type of *P. citricola*, differing in the ITS region by 7 bp. However, there appear to be many subclades within the *P. citricola* complex which may correspond to additional new taxa. Further study of this important species complex is required to elucidate the host and geographic range and phylogeny of isolates within the complex and to determine if they constitute new species.

In GenBank, 11 ITS sequences, designated as *P. citricola*, are identical to *P. multivora*. Seven are from unpublished studies in Hungary, Canada, Switzerland, Korea and Japan, and two sequences are from isolates of Moralejo et al. (2008) from ornamental nurseries in Spain, an isolate from *Mangifera indica* in Spain (Zea-Bonilla et al. 2007). In addition, an isolate designated as *P. sojae* in a study from Japan also has identical sequence (Villa et al. 2006). This low number of very recent submissions of *P. multivora* sequences as compared to the high number of other sequences from the *P. citricola* complex indicates that *P. multivora* may have been introduced to these countries. Due to the widespread distribution of *P. multivora* across natural ecosystems in WA, it is likely that WA may be a source of dispersal, possibly via the nursery trade (Brasier 2008).

In the *cox*1 analysis *P. multivora* and the ex-type isolate of *P. citricola* grouped together with other ITS clade 2 species, although the distance between *P. multivora* and *P. citricola* was greater in the *cox*1 analysis than in the ITS analysis. In the ITS sequence there was only 1bp difference between all isolates of *P. multivora*. In the *cox*1 analysis there were more differences resulting in the formation of subclades. The greater phylogenetic variation and presence of subclades in the *cox*1 analysis reflects the expected faster rate of mitochondrial than genomic DNA evolution (Kroon et al. 2004). The observed variability, however, strongly supports the hypothesis that *P. multivora* in WA is not a recent clonal introduction, but rather was introduced long ago or is endemic. This is also reflected by the phenotypic variability observed among isolates of *P. multivora*. There was generally some variation in the colony growth patterns and growth rates, and in the dimensions of morphological structures of the different *P. multivora* isolates. However, isolate WAC13204 was particularly different from the other five isolates of *P. multivora*, having significantly larger sporangia, a higher maximum growth temperature and faster growth rates.

A *cox*1 sequence for *P. citricola* was available on GenBank from the study of Kroon et al. (2004). In their study, this putative *P. citricola* was closest to *P. cryptogea* and they discussed this incongruency, as it was one of the few species that did not fall into the same clades in both the mitochondrial and nuclear gene analysis. With our new sequences for *P. citricola*, including the ex-type isolate, it is clear that the sequence used for *P. citricola* by Kroon et al. (2004) was incorrect.

Morphological similarities between taxa, as observed between *P. multivora* and *P. citricola*, are increasingly found in the unravelling of different species complexes within the genus *Phytophthora* using molecular methods (Brasier et al. 2003, 2004, Jung et al. 2003). This study therefore highlights the importance of using ex-type cultures where available and the value of using molecular tools to unravel the ambiguity of species previously identified solely on morphological characteristics. Over the last 30 yr, in the absence of sufficient molecular techniques, *P. multivora* has been routinely identified in the south-west of WA as *P. citricola* using morphological characteristics. Similar misidentification of *Phytophthora* species has occurred with the identification of *P. pseudosyringae* isolates as *P. syringae* (Jung et al. 2003).

Despite the similarities, there are clear morphological and physiological differences between *P. multivora* and the ex-type isolate of *P. citricola*. If more isolates of *P. citricola* were to be examined the morphological differences between the two species may be less resolved. *Phytophthora multivora* and *P. citricola* produce different colony growth patterns on V8A, MEA and CMA with the most distinct variation observed on V8A. *Phytophthora multivora* has a clear optimum growth temperature of 25 °C while the optimum growth rate of *P. citricola* is at 22.5 °C and decreases by only 0.2 mm/d between 22.5–30 °C. Over the whole temperature range, except of the optimum temperature of 25 °C, *P. multivora* isolates are slower growing than *P. citricola*. Sporangial shapes of *P. multivora* are generally more uniform while in *P. citricola* sporangia are more variable and the frequency of distorted shapes is significantly higher. A high variability of sporangial shapes was also found by Zentmyer et al. (1974) studying *P. citricola* isolates from *Persea americana* in California. Although most morphological measurements of the ex-type isolate of *P. citricola* fell within the range of *P. multivora* isolates there were clear differences between both species. All six *P. multivora* isolates produced on average significantly smaller oogonia and oospores, and significantly thicker oospore walls than *P. citricola*. This is reflected by the oospore wall index, which is the ratio between the volume of

the oospore wall and the volume of the entire oospore (Dick 1990). The oospore wall index of *P. multivora* (0.52 ± 0.07) was almost 50 % higher than that of the ex-type isolate of *P. citricola* (0.36 ± 0.05). A calculation of the oospore wall index using the original datasets of Jung et al. (1999, 2002, 2003) for *P. europaea* (0.37 ± 0.07), *P. ilicis* (0.41 ± 0.11), *P. pseudosyringae* (0.27 ± 0.09), *P. psychrophila* (0.42 ± 0.06), *P. quercina* (0.45 ± 0.08), *P. syringae* (0.24 ± 0.07) and *P. uliginosa* (0.46 ± 0.09) demonstrated that *P. multivora* had the highest oospore wall index of all nine species examined. The thick oospore wall of *P. multivora* is most likely an adaptation to the seasonally extremely dry soil conditions in WA. This survival mechanism was also suggested for *P. quercina* in European oak forests (Jung et al. 1999, 2000). After 4 wk in V8A at 20 °C, in all six *P. multivora* isolates, more than 90 % of the oospores had germinated directly. This lack of dormancy had previously been observed for oospores of *P. medicaginis* (Erwin & Ribeiro 1996), however, this does not preclude dormancy occurring under different conditions. No direct germination was observed in cultures of the ex-type isolate of *P. citricola* growing under the same conditions. This result corresponds to the low oospore germination rates observed in European isolates of the *P. citricola* complex (Delcan & Brasier 2001). Whether these differences in germination rates reflect different survival mechanisms of the two species requires further investigation.

Phytophthora multivora can easily be distinguished from other homothallic *Phytophthora* species with paragynous antheridia and semipapillate sporangia by its unique combination of morphological and physiological characters, and DNA sequences. *Phytophthora multivora* is separated from *P. syringae* by the absence of hyphal swellings, the occurrence of distorted and bipapillate sporangia, thicker oospore walls, different colony growth patterns on V8A, MEA and CMA, higher optimum and maximum temperatures for growth, and different ITS and *cox*1 sequences (Waterhouse & Waterston 1964, Erwin & Ribeiro 1996, Jung et al. 2003). *Phytophthora multivora* can be distinguished from *P. pseudosyringae* by the absence of hyphal swellings and caducity of sporangia, the occurrence of distorted bipapillate sporangia, thicker oospore walls, different colony growth patterns on V8A, MEA and CMA, higher optimum and maximum temperatures for growth, and different ITS and *cox*1 sequences (Jung et al. 2003). *Phytophthora multivora* is discriminated from the original *P. inflata* of Caroselli & Tucker (1949) by having larger sporangia, markedly smaller oogonia and thinner oospore walls, and by the absence of inflated irregular antheridia which are often twining or twisted around the oogonial stalk in *P. inflata*.

Under the original morphological identification as *P. citricola*, an isolate of *P. multivora* was used in an underbark inoculation test, and caused significantly longer lesions on stems of *E. marginata* and *C. calophylla* than *P. cinnamomi* (Shearer et al. 1988). Experiments are currently underway to determine pathogenicity of *P. multivora* towards *E. gomphocephala* and *E. marginata*.

Phytophthora multivora has been isolated in WA from natural forest and heath-land stands for the last 30 yr from beneath dead and dying plants of 16 species from seven families. *Phytophthora multivora* is very widespread in south-west WA with a distribution similar to that known for *P. cinnamomi*. The VHS uses detection methods developed specifically for *P. cinnamomi* and even under these conditions, *P. multivora* is the next most commonly isolated taxon after *P. cinnamomi*. There is now evidence that in some sites it may be *P. multivora* and not *P. cinnamomi* that is responsible for tree mortality, while the latter is driving the collapse of whole ecosystems known as *Phytophthora* dieback. These findings may have direct implications for forest management and biosecurity, and our study highlights the potential importance of new and yet undescribed *Phytophthora* taxa in natural ecosystems in the south-west of WA (Burgess et al. In press), and the need for continued research.

Acknowledgements The authors are grateful to Murdoch University for a Murdoch University Research Scholarship for the first author, the Australian Research Council and industry partners for generous financial support (LP0346931 and LP0668195), Janet Webster and Juanita Ciampini (VHS) for providing *P. multivora* isolates for DNA sequencing, Diane White (CPSM) for the sequencing of isolates, Amanda Hewson (CPSM) for assistance in field work, Dang Tan Dang (CPSM) for assistance in morphological and physiological measurements, Alex Rea (CPSM) for providing the *cox*1 sequence of the VHS isolates, and Bernard Dell, William Dunstan, Daniel Hüberli (CPSM), and Chris Dunne and Colin Crane (Deptartment of Environment and Conservation, WA) for constructive discussions.

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